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TRANSLOCATION OF 2-METHOXY-3,6-DICHLOROBENZOIC ACID AND 4-AMINO-3,5,6-TRICHLOROPICOLINIC ACID IN CANADA THISTLE (CIRSIUM ARVENSE (L.) SCOP.)

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Translocation of 2-Methoxy-3,6-dichlorobenzoic Acid and 4-amino-3,5,6-trichloropicolinic Acid in Canada Thistle (Cirsium arvense (L.) Scop.)", submitted by Fa-Yan Chang in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Translocation of 2-methoxy-3,6-dichlorobenzoic acid (dicamba) and 4-amino-3,5,6-trichloropicolinic acid (picloram) in Canada thistle plants was studied by physiological responses, bioassay, autoradiography and radioassay methods. Both these herbicides were readily absorbed either by foliage or by roots, and translocated in the phloem and xylem. After application to the leaves they were also exuded from roots of the treated thistle plants into soil in amounts sufficient to affect safflower seedlings growing in the same soil. Locus of application greatly affected the rate and extent of the translocation whereas the effect of stage of plant growth on the transport of these herbicides was not obvious. When applied on the midrib 5 cm from the base of the leaf blade it took about six hours to translocate injurious amounts of the herbicides from the treated spot to the stem.

Quantitative determinations made by bioassay showed that picloram was distributed throughout the plant within one day following application on the upper surface of one centrally located leaf. The amount of picloram present in other parts of the plant no longer increased after one day while it was still increasing in the treated leaf. Total recovery of this herbicide decreased rapidly with time after application. The results of radioassay indicate that distribution of dicamba in thistle plants follows a pattern similar to that of picloram. Within three hours a small amount of the dicamba label was translocated from the treated leaf to other parts of the plant and the radioactivity recovered from these plant parts reached a maximum three days after application. A major portion of the recovered amounts of these chemicals

remained in the treated leaf.

Autoradiograms showed that, following foliar application, dicamba tended to accumulate in the young developing leaves in the shoot apex and in the new sprouts. When absorbed by the roots, it was distributed rapidly throughout the plant with a little accumulation in the young leaves.

One C^{14}-metabolite was detected in the plant extract in addition to the unaltered dicamba. This metabolite was found mainly in the treated leaf and its proportion in terms of recovered radioactivity increased from 3.4% in one day to 36.9% 54 days after application.

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INTRODUCTION

Canada thistle (Cirsium arvense (L.) Scop.) is a troublesome perennial weed reproducing by seeds and creeping roots (3, 35). Its troublesome nature is mainly due to its deep root system. Since a small piece of the root can give rise to a new plant, reproduction and spread can be very fast. Once the weed has been established, it is difficult to eradicate, because most of the commonly used herbicides can not reach the deep root in a sufficient amount to kill the underground meristematic regions. The most widely used herbicide 2,4-D, for example, is effective in controlling many broad-leaved weeds. Its translocation in the plant is limited, however, and it will frequently kill only the top-growth of Canada thistle without eradicating the plant.

In recent years two chemicals, 2-methoxy-3,6-dichlorobenzoic acid (dicamba) and 4-amino-3,5,6-trichloropicolinic acid (picloram), have been found effective in the control of this species and some other deep-rooted perennial weeds. This effectiveness is thought to be related to their mobility in the plant. However, little is known about their translocation behaviour.

The experiments reported here were designed to assess the importance of the locus of application of these chemicals and the growth stage of the plant with respect to their translocation, the time required for an amount of these chemicals sufficient to cause injury to be exported from the treated leaf to the rest of the plant, the extent of root exudation, and the distribution of these herbicides in the plant following application to the leaf or to the roots.

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LITERATURE REVIEW

Translocation of herbicides

There are two major systems in the plant which are involved in translocation (38). The conduits in the woody tissue, or xylem, consist of thick-walled open vessels of dead cells through which water and mineral nutrients move to the upper regions of plants after being taken up by the roots from the soil. The conduits through which products of photosynthesis and other organic nutrients travel from the leaves to growing regions of roots and shoots consist of the sieve tubes of the phloem.

Transport of materials may occur in either the living (symplast) or non-living (apoplast) parts of the plant. These terms were introduced by Münch in 1930, according to Leonard (26), with symplast designating the sum total of interconnected living protoplasm of the plant and the apoplast the non-living continuous cell wall phase around the symplast. The symplast is connected from cell to cell by plasmodesmata connections, or tubules. The sieve tubes are considered a part of the symplast, while the tracheids, xylem vessels, cell walls and intercellular spaces are regarded as the apoplast.

The upward transport through the xylem in the transpiration stream is considered to be due mainly to the reduced pressure resulting from the evaporation of water from the mesophyll (6). Translocation in the symplast is more complicated. Although several theories have been proposed to explain the mechanism of translocation in this living conduit, no one of them has ever been generally accepted (38). However, over ten years of work with radioactive herbicides, Crafts and Yamaguchi (10)

concluded that systemic distribution of foliage-applied compounds follows a source-to-sink pattern, from the regions of synthesis of foods to regions of their utilization, indicative of movement en masse of the assimilate stream via the phloem. The consistent bypassing of mature leaves, the high concentrations in young growing shoot tips, root tips, and intercalary meristems, and the reversibility of flow brought about by proper manipulation, all indicated a mass-flow type of mechanism.

Following the source-sink pattern, symplastic translocation in plants is variable, depending upon the relative position of the sources and sinks of the plant (4, 39). Lower leaves export primarily to the roots, the upper leaves to the stem apex, and the intermediate ones in both directions.

In general, the translocation of herbicides follows a pattern similar to that of most other organic compounds, and even some inorganic compounds. The rate of translocation and pattern of distribution depend on many factors, such as the environment, the plant species and stage of development as well as the position of application (7, 33). The herbicides themselves contribute a great deal to the variation of the translocation pattern.

Herbicides can be divided into several categories on the basis of translocation characteristics (26): (a) those that move slightly if at all, e.g., oil and oil-soluble esters of 2,4-D and 2,4,5-T, diquat; (b) those that are translocated in the symplast primarily, e.g., the acid and salt forms of 2,4-D and 2,4,5-T; (c) those that are translocated in the apoplast only, e.g., fenuron and other substituted ureas and the symetrical triazines; (d) those that move in both the symplast and apoplast, e.g.,

amitrole, maleic hydrazide. Symplastic movement is of greatest importance following leaf application, while apoplastic movement is of greatest importance following root application. Compounds possessing the ability to move in either the symplast or the apoplast have the greatest potential as herbicides (26). Some compounds may leak from root into the culture medium, examples being 2,4-D, MCPA, 2,3,6-TBA, dicamba, and picloram (9, 22, 29, 30, 31, 34).

2-Methoxy-3,6-dichlorobenzoic acid

2-Methoxy-3,6-dichlorobenzoic acid, with the common name dicamba and the commercial name 'Banvel D', was first introduced for field testing during 1961 (8). The acid is only slightly soluble in water; the dimethylamine salt formulation, however, is readily water-soluble. It has been used with success for the control of Canada thistle and has shown great potential as a selective herbicide for the control of other broad-leaved weeds such as green smartweed, wild buckwheat, and Tartary buckwheat in cereal grains (8, 13, 23, 40).

The results of leaching studies of Friesen (14) and Vanden Born (41) suggest that dicamba moves readily in the soil with added water. Harris (18) reported that dicamba moves upward as well as downward with the movement of the water front.

Using Tartary buckwheat bioassay Friesen (14) found that dicamba persisted longer in soil than did 2,4-D. In not-autoclaved soils the buckwheat plants showed no injury from the 2,4-D at 16 oz/acre after two weeks, but nearly all of the plants were killed by dicamba at 8 oz/acre after twelve weeks of incubation.

Limited available information suggests that dicamba is translocated readily in plants. Hodgson (20) reported that surrounding bracken plots sprayed with dicamba in the previous year there were many deformed fronds, in many instances several feet from the nearest treated areas, indicating that the compound or some derivative had been translocated over far greater distances than he had ever observed for any other chemical so far tested for the control of bracken.

Using C^{14}-labeled chemicals, Leonard et al. (28) observed that dicamba moved readily from the roots to the shoots in grape cuttings. With foliar application, the label also appeared in the root. Linder (31) reported that, following foliar application to Pinto bean plants, a detectable amount of dicamba was exuded from the root into the surrounding medium. The root exudate was chromatographically identical with the applied compound.

4-Amino-3,5,6-trichloropicolinic acid

The chemical 4-amino-3,5,6-trichloropicolinic acid, under the common name picloram and the trade name 'Tordon', was introduced as an experimental herbicide by the Dow Chemical Co. in 1963. It is formulated as the potassium salt or the triisopropanolamine salt, which are readily soluble in water.

Hamaker et al. (17) first reported the herbicidal properties of picloram. They stated that, except for mustards, it is more toxic to many broad-leaved plants than are 2,4-D and 2,4,5-T, and that it is comparable to 2,4-D in absorption by foliage, in translocation, and in soil-leaching characteristics. In soil, however, it retains the activity for a longer

time. Since 1963, a number of reports have indicated that picloram offers considerable promise for the control of deep-rooted perennial weeds (2, 24, 43) and some woody species (5,44, 45, 46). Alley (2) has reported that picloram seems especially active towards Canada thistle. Laning (24) indicated that picloram is highly active when applied to foliage of Canada thistle and field bindweed, but in California experiments it has been more effective when leached into the root zone.

Though picloram is more toxic to broad-leaved plants than to grasses, it also shows potentiality for the control of some grassy species (1, 41). Uptake of this chemical by some grasses through roots or rhizomes is more effective than foliar uptake (41).

The persistence of picloram in soil has been reported. Hamaker et al. (17) reported that 568 days after application of picloram to soil it retained 50 per cent of its effectiveness. Using a safflower bioassay, Goring et al. (16) observed that, under field conditions at a number of locations in the western United States, losses of picloram ranged from 58 to 96 per cent within one year and from 78 to 100 per cent within two years after application. Estimated half-lives for picloram at the various locations ranged from one to thirteen months. Picloram was not found below depth of 48 inches and, in most instances, the highest concentrations were in the top 12 inches. A recent report of Merkle et al. (32) indicated that, in sandy soils with relatively high rainfall, picloram was detected to a depth of 24 inches within six weeks after application. No detectable residues were found in the upper 24 inches of soil after one year with rates as high as 8 lb/acre.

Few studies on the translocation of picloram in plants have been

reported. Leonard et al. (27) concluded that, on the basis of physiological responses, in grape cuttings picloram was translocated in a manner identical to that of dicamba from root application. In a tree injection experiment Watson and Mesler (44) observed that picloram was translocated readily both upward and downward from the point of application; lateral translocation in the tree was limited. Hurtt and Foy (22) presented evidence that picloram, as well as dicamba, was foliarly-absorbed, phloem-translocated, and excreted from the roots of bean plants.

MATERIALS AND METHODS

General

Experiments were conducted in the greenhouse, and in the field at the University Farm at Ellerslie, during 1964 and 1965. The formulations of the herbicides used were the dimethylamine salt of dicamba and the potassium salt of picloram, unless otherwise stated. To avoid genetic variability, Canada thistle plants used in these experiments were all vegetatively propagated from a single plant. Each plant was grown from a single creeping root section about 8 cm long. All leaf applications were made in water solution containing the wetting agent Tween 20 at a concentration of 0.1% to facilitate penetration.

In the greenhouse, the temperature was maintained at approximately 20°C. during the winter. During the summer, however, temperatures in excess of 35°C. were occasionally experienced. Supplementary lighting was given during the winter months to keep the day-length at 16 hours. The plants were grown in 15-cm plastic pots and treated usually 6 - 7 weeks after planting, when they were 10 - 14 cm high with 10 - 15 leaves on the main shoot. Treated leaves were approximately at the mid-point on the stem.

In the field creeping root sections were planted at 2-meter intervals, while the surrounding area was kept free from other weeds. After becoming established, the plants were treated by foliar application of dicamba and picloram or by soil application of granular picloram.

After application of the herbicide, the plants were observed for the development of visible symptoms on the treated parts and on other

portions of the plants. The appearance of epinastic responses such as swelling, curvature and bending of stems, and chlorosis of the young leaves, was taken as evidence of the transport of the herbicides to the organs of the plant showing such symptoms.

Preparation of extracts for bioassay

Canada thistle plants were treated with 1,000 µg of picloram by applying 100 µl of the chemical solution to a plastic ring (diameter 8 mm) which had been cemented to the upper surface of a full grown leaf with lanolin. The ring was then covered with a piece of parafilm to prevent rapid evaporation of the treatment solution. The plants were harvested 1, 3, and 9 days after application. Duplicate plants were used in each treatment. The residue of picloram on the leaf surface was washed off in 20 ml of 50% ethanol. Then the plants were separated into several parts and each part was ground in 95% ethanol in a Waring blender. Flasks with ground material were kept at room temperature for twelve hours. The preparations were then filtered using a Buchner funnel and Whatman No. 1 filter paper. The extracts and the leaf washing were evaporated to near dryness under reduced pressure in a rotary evaporator at 38 - 42°C. and then brought to a volume of 4 ml by adding 50% ethanol. If the herbicide concentrations of certain extracts were too high, on the basis of a preliminary test, they were further diluted to bring them into the normal range for sunflower bioassay.

Autoradiography

Translocation of C^{14}-carboxyl-labeled dicamba (dicamba*)[1] in thistle

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[1] Obtained from New England Nuclear Corporation through the courtesy of Velsicol Chemical Corporation.

plants following foliar or root application was studied by the methods described by Crafts et al. (10, 37, 47).

Ten µl of a solution of dicamba* (specific activity 1.89 mc/mM) in 50% ethanol was applied in a lanolin ring on the midrib of a full-grown leaf of a thistle plant with two shoots. The concentration used was 0.1 µc/10 µl (approximately 12 µg of dicamba*). The treated plants were left in the greenhouse for 2, 4, 8, 16 hours, 1, 2, 4, and 8 days. At the end of the treatment periods the lanolin paste was removed and the treated spots were covered with a piece of masking tape to prevent radiocontamination. Duplicate plants were harvested, freeze-killed with crushed dry ice, and freeze-dried. Freeze-drying was carried out using a vacuum tank equipped with a moisture-trapping flask placed in the vacuum line and bathed in a mixture of acetone and dry ice. The drying process was completed in 2 - 3 weeks. The dried plant materials were humidified, mounted and flattened, and then autographed for three weeks using Ansco non-screen safety x-ray film. Films were developed in Ansco Liquadol developer for four minutes and fixed for ten minutes or longer in Kodak fixer.

When translocation of the chemical following root absorption was studied, the plants were grown in soil and transferred four days before treatment to one-half strength Eliasson nutrient solution (11) in quart jars wrapped with aluminum foil. For treatment the plants were further transferred to 250-ml beakers containing 100 ml nutrient solution with 0.5 µc of dicamba* (concentration 0.6 µg/ml). The plants were harvested ½, 1, 2, 4, 8, 16 hours, 1, and 4 days following treatment. At harvest the roots were rinsed under running tap water for three minutes. Freeze-

killing, drying, and autoradiography were carried out following the same procedures as in foliage treatment.

Determination of radioactivity in extracts

Distribution of dicamba* in the plants following foliar application was investigated by determining the radioactivity in extracts of different parts of the plants.

Canada thistle plants with two shoots were treated with dicamba* in 50% ethanol by droplet application in a plastic ring on a single leaf. The ring was covered with a piece of parafilm after application. At harvest the residue of dicamba* on the leaf surface was washed off in 20 ml of 50% ethanol. The plants were cut into several parts and the separated plant parts were stored in a freezer at -20°C. until they were extracted.

The plant parts were ground and extracted in 95% ethanol overnight. After filtration the extracts were evaporated to 5 - 10 ml. Aliquots of 0.5 ml of the concentrated extracts were applied to aluminum planchets and dried on a hot plate. If the radioactivity on the planchets was too high to be counted effectively, the extracts were further diluted to keep the radioactivity below 3,000 counts per minutes (cpm) per planchet. To each planchet 50 μl of 1% Tween 20 was added to ensure even distribution of the extracts and thus to increase the accuracy of counting. Radioactivity of the extracts was then determined using a Nuclear-Chicago Model D-47 gas flow counter, which had an efficiency for C^{14} of approximately 33% and an average background of 18 cpm. Corrections were made for background and aliquot fraction.

RESULTS AND DISCUSSION

I. Effect of point of application on translocation

To investigate the importance of the locus of application on translocation, plants grown in the greenhouse were treated with dicamba and picloram at 10 - 1,000 µg/plant (acid equivalent) in 50 µl solution on four different positions, i.e. (a) as a droplet in a plastic-tube-and-lanolin ring on the midrib of a full-grown leaf approximately one-third the length of the leaf blade from the stem, (b) as droplets on the midribs of four leaves, (c) on the growing tip, and (d) injected in the soil near the stem.

Visible stem bending usually began ten hours after application of the herbicides to the leaf or to the soil. The degree of stem bending varied from 15 to more than 90 degrees, depending on the dosage applied and the point of application (Fig. 1).

Necrotic spots usually occurred on the treated area one to three days following leaf application. With time necrosis sometimes extended to the entire treated blade and to young leaves in the growing tip. Gradually formative effects and chlorosis appeared on the untreated shoots. Fig. 2 shows the plants seven weeks following application of 100 µg of picloram. All the treated leaves and the young leaves in the tip of the treated shoots died. Injury on the untreated shoots was apparent. The plant in the soil treatment died, but the plant treated on the growing tip was still able to flower on the untreated shoot.

Swelling of the growing tip as shown in Fig. 3 usually occurred on the treated shoot some seven to ten days following treatment; swelling

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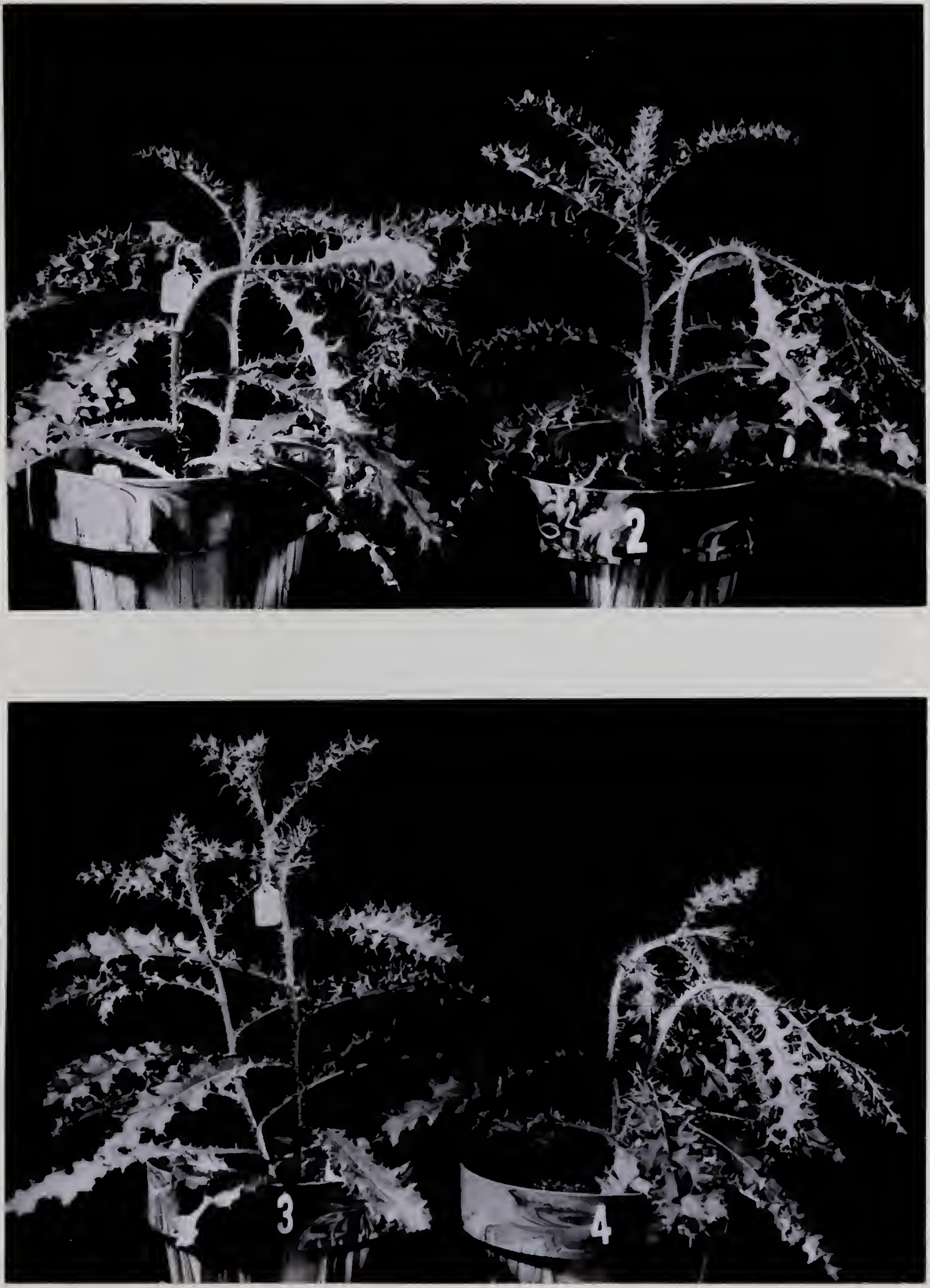


Fig. 1. Canada thistle plants 20 hrs. after treatment with 1,000 μg picloram showing stem bending.
Upper left: 1-leaf application.
Upper right: 4-leaf application.
Lower left: growing tip application.
Lower right: soil application.

Fig. 2. Canada thistle plants seven weeks after application of 100 μg of picloram per plant showing effect of locus of application. Upper left: 1-leaf application. Upper right: 4-leaf application. Lower left: growing tip application. Lower right: soil application.

Fig. 3. Swelling of Canada thistle growing tip as a result of treatment with 50 µg dicamba on a single mature leaf. Regrowth from the swollen tip occurred after a period of stunting. Photographed 47 days after treatment.

of the growing tip on untreated shoots, if any, appeared one to two weeks later. This swelling symptom was particularly severe in dicamba treatment, though it also occurred in the picloram-treated plants. When relatively high concentrations were applied, death of the growing tip occurred, instead of swelling.

All of these symptoms on different parts of the plants indicated that sufficient herbicide was transported from the points of application to the rest of the plant to result in injury with a tendency for the herbicide to accumulate in the meristematic regions - at least the injury symptoms were most severe in those regions.

Most rapid appearance of injury symptoms and most severe symptoms occurred following application of the herbicides to the soil near the stem. Application to four leaves caused more rapid and serious injury than did applying the same dosage in a ring on a single leaf, probably due to the larger absorption area and enhanced translocation in the four-leaf application. Application to the growing tip resulted in much slower appearing and less serious injury. This suggests that export of leaf-applied dicamba or picloram is dependent upon the export of photosynthate which has not yet been built up in sufficient amount for exporting in the very young leaves at the growing tip.

Vanden Born (42) observed the effect of concentration of dicamba on translocation of this chemical in Tartary buckwheat. In 10 µl droplets applied on a single leaf 20 µg of dicamba brought about the greatest injury to the buckwheat seedling; with further increasing concentration injury at first remained nearly the same, then fell off. In the present work, however, no limiting effects of high dosages were observed on Canada

thistle over a dosage range of 10 - 1,000 µg applied in 50 µl solution, though higher concentration caused more severe contact injury at the point of application, which may restrict the export of the applied chemical from the treated area. The injury symptoms on the untreated shoot as well as on the treated shoot became more severe as the dosage (concentration) increased (Fig. 4). This result indicates that, though injury may restrict the translocation to a certain extent, the total amount of herbicide transported out of the treated leaf increases with dosage over this range.

Fig. 4. Growing tips of Canada thistle shoots treated with dicamba showing effects of dosage. Dicamba was applied in a 50 µl droplet on a single leaf. From left to right: 10 µg, 100 µg, 1,000 µg (growing point died). Photographed one month after treatment.

In comparing the two herbicides, it was observed that less necrosis occurred on the treated area in picloram treatment than in dicamba treatment. Dicamba caused more rapid appearance of symptoms on the untreated shoots than did picloram whereas the latter caused more severe injury to the treated ones. Picloram appeared to be more toxic

than dicamba at equivalent dosage, especially in experiments of long duration, but the transport of picloram to untreated shoot seemed to occur less readily than did that of dicamba. In addition, the roots of the plants which received the picloram treatment on the leaves showed various degrees of swelling, becoming up to six times as thick as the normal ones (Fig. 5). When the swollen root was sectioned and stained with phloroglucinol it was observed that the vascular tissue was distributed very irregularly (Fig. 6).

From the fact that the untreated shoots of the dicamba-treated plants were affected, it may be concluded that a certain amount of this chemical also moved downwards into the roots. In no case, however, were any injury symptoms observed on the roots of the plants treated with dicamba. It is possible that the creeping roots of Canada thistle serve only as a channel of transport in the case of dicamba whereas picloram can accumulate there in amounts sufficient to cause swelling. On the other hand, the observations may also simply reflect the difference in sensitivity of the root to the two herbicides.

When the swollen roots were replanted three months after treatment, no sprouts emerged from the root sections showing severe swelling, but those with only slight swelling did grow and no injury symptoms were observed on the new sprouts. However, when the treated plants were left intact, any new sprouts produced did show injury symptoms. This suggests that, in those root sections, there was not enough freely movable herbicide to cause injury to the new sprouts; when the plants remained intact, however, the chemical was translocated from other parts of the plants to the new sprouts in sufficient amounts to cause visible symptoms, indicating

Fig. 5. Swollen root of Canada thistle plant treated with 1,000 μg picloram on a single leaf (left), and a normal root section of an untreated plant (right). Photographed three months after treatment.

Fig. 6. Cross sections of Canada thistle roots showing disturbed vascular system of a swollen root caused by picloram treatment (upper, X 3), and regular arrangement of vascular system of a normal root (lower, X 20).

that redistribution of the herbicide occurred in the plants even three months after treatment.

II. Effect of stage of plant growth

The effect of stage of plant growth on translocation was studied by applying the herbicides at three stages: (a) young plant well before budding (July 7, 1964; July 19, 1965), (b) early bud stage (July 28, 1964; August 4, 1965), and (c) early flowering stage (September 3, 1964; September 5, 1965). This experiment was conducted in the field. The herbicides were applied at dosages of 50 to 2,000 µg/plant by placing the chemical solution in a ring on a single leaf or by spraying the treatment solution over a single shoot. When applied in the ring, the herbicide concentration remained constant at 20,000 ppm and the dosage was adjusted by varying the volume of application. Spraying was carried out with a fine atomizer using 2 to 4 ml of spray solution of various concentrations. The surrounding ground and the other shoots were covered with polythene sheeting to prevent spray contamination.

Following application of the herbicides, injury symptoms occurred on the treated shoot and the untreated shoots, similar to the symptoms observed in the greenhouse. The symptoms were most distinct on young shoots that emerged after treatment, even if they were as far as one meter from the treated one.

The response to treatment at the early bud stage was about the same as that at the young plant stage. When treatment was made at the flowering stage, the injury symptoms on old shoots were not so obvious, but injury to the young sprouts was clearly observed, indicating that trans-

location of the herbicides from the treated spot to young shoots had taken place. The reduced response on the old shoots might have been due to the absence of actively developing tissue which is usually the locus for herbicide accumulation and/or action.

III. Soil application of granular picloram

When Canada thistles were at the young plant stage with 10 - 15 leaves, picloram in a granular formulation (2 per cent active chemical as the potassium salt on clay granules) was applied on the soil surface at distances of 0, 2.5, 5, 10 and 20 cm from the main shoots of the plants and at dosages of 2 - 16 mg/plant. Except for the 0 cm treatment, in which the granules were placed at the base of the main shoot, the chemical was applied as a circular band at specified distances around the main shoot. In another test, picloram granules were applied on the soil surface in different patterns, i.e. one-side, two-side, and circle applications, all at the same distance from the shoot.

When application was made close to the main shoots, most of the plants were completely killed. In the 2.5 cm and 5 cm treatments, the main shoots died, and some other shoots were slightly to seriously affected. In the 10 cm treatment, however, only the treated main shoots showed slight chemical injury. The higher the rate of application, the more severe the injury to the plants, but, at all rates, there was no visible effect to the plants when the chemical granules were placed 20 cm from the plant. Apparently there was little lateral movement of the herbicide in the soil.

Excavation of roots showed that the roots of the dead plants died

and rotted. In the roots of the partially dead plants only a short section just beneath the chemical rotted off, thus separating the plant into several individuals.

The greater the number of locations of application (one side, two sides, or circular band), the more severely the plants were injured. This result agrees with the finding of Wiltse (46) that in brush control a high concentration of granular picloram over a portion of the plant root was less effective than a lower concentration spread uniformly over the root system. Wiltse also observed that injury to the plant occurred only on the side where the herbicide had been applied. He suggested that some mechanism may prevent absorption by the root of amounts of herbicide lethal to the whole plant when only a relatively small portion of the plant roots is in contact with the herbicides. I think it is due to damage to the transport system of the roots which are in contact with the chemical.

IV. Movement of the herbicides in root sections

Dicamba and picloram were applied to one end of 8-cm root sections in 10 µl droplets of different concentrations to give dosages of 0 to 200 µg/section. The root sections were inserted vertically in wet vermiculite to keep moist, with a small portion exposed to the air. A plastic ring of 3 mm inside diameter was placed on the exposed end and the treatment solution was applied in the ring. About 24 hours following application, when the treatment solution had been absorbed, six of the sections from each treatment were planted in two 15-cm pots and allowed to grow in the greenhouse. Treatment effects observed included delayed sprouting and decreased growth vigor, and also injury symptoms on the shoots produced.

Sprouting was increasingly inhibited and delayed with increasing herbicide dosage (Table I). Injury symptoms appeared on the shoots produced in dicamba 5 µg and picloram 1 µg treatments. Picloram inhibited sprouting at least three times as effectively as did dicamba.

Table I. Sprouting of Canada thistle root sections following treatment with dicamba or picloram

Dosage (µg/section)	0	1	2	5	10	20	50	100	200
					Dicamba				
2 weeks	6	2	2	4	1	0	0	0	0
7 weeks	9	5	6	6	5	4	2	2	0
					Picloram				
2 weeks	6	3	0	0	0	0	0	0	0
7 weeks	9	5	1	0	1	0	0	0	0

Each figure in the table is the number of sprouts from six root sections.

These results indicate that a certain amount of the herbicides had moved from the point of application into the root section before sprouting occurred. Since there is little translocation of assimilates and no transpiration stream in the sections, both of which are believed to be associated with the translocation of herbicides, the movement of dicamba and picloram in the root sections must follow a mechanism which is different from that in the actively growing plants; simple diffusion is the most likely mechanism.

When the plants were dug out for observation at the end of the experiment, it was found that most of the sprouts were located at positions far from the treated ends of the root sections, and the root sections more or less rotted starting from the treated ends. How much of the applied herbicides actually reached the unrotted portion of the root sections is not known. The differences in response to dicamba and picloram shown here probably simply reflect the relative toxicity of the two herbicides rather than any differences in their mobility in the root sections. The finding that picloram is much more toxic than dicamba to Canada thistle roots is similar to that of Hull (21), who observed that, to Johnsongrass rhizomes, picloram was more toxic than dicamba.

V. Gradual mutilation experiments

In a series of experiments the time required for a distinctly phytotoxic amount of dicamba or picloram to be exported from the treated leaf was investigated. The herbicides were applied as a single droplet to the midrib 5 cm from the base of the blade, and the treated leaf was removed at different time intervals following application.

In a preliminary test dicamba was applied at dosages of 100 and 1,000 µg/plant. When the treated leaf was cut off up to four hours after treatment, no significant effects on the plants were observed. If the treated leaf was allowed to stay on the plant for eight hours, slight stem bending occurred and formative effects appeared on the young leaves of the treated shoot (Fig. 7). If the treated leaf was cut off 24 hours following application, the treated shoot later showed swelling of the growing tip, and injury symptoms appeared on the untreated shoots. In the 48-hour treatment the effects on the treated shoot were almost the

Fig. 7. Parts of Canada thistle shoots showing the time-course of translocation of dicamba as indicated by gradual mutilation. Following foliar application of 1,000 μg of dicamba, the treated leaf was removed at different time intervals. From left to right: control, 8 hrs., 24 hrs., 48 hrs., and no removal. Photographed one month after treatment.

same as those on the plant which was left intact; growing tips of these shoots died and growth was stunted. The effects on the untreated shoots in the 48-hour treatment were, however, still less than those on the plants with the treated leaf left intact.

In another experiment both dicamba and picloram were applied at the rate of 100 µg/plant, and an additional six-hour period was included in the time series. All the dicamba treatments showed the same results as in the first experiment, except that slight injury symptoms appeared on plants with their treated leaves removed six hours after application of the herbicide. Plants treated with picloram showed the same response to the mutilation as those treated with dicamba, but effects on the treated shoots were more marked and effects on untreated shoots were delayed.

All these observations suggest that up to four hours following application of dicamba and picloram no significant export of these herbicides from the treated leaf took place; it took about six hours for an injurious amount to be translocated from the treated spot to the stem. Within 24 hours, a sufficient amount was transported to the shoots to cause serious injury to the plants, and the exporting of the herbicides continued even after 48 hours.

If the treated spot was washed with water, instead of removing the entire treated leaf, at one or two hours after application of the herbicides, all the plants showed distinct injury symptoms, indicating that a certain amount of the chemicals had already penetrated into the leaf tissue within such a short period.

VI. Root exudation following foliar application

In this experiment an attempt was made to show whether dicamba and picloram applied to the leaves of Canada thistle plants could be exuded from the roots into the surrounding soil. The experiment was conducted in the greenhouse employing a picloram-sensitive plant, safflower (16), to indicate the presence of exuded chemical.

Dicamba and picloram at dosages of 200, 800, and 2,000 μg were applied as droplets to the leaves of thistle plants grown in 15-cm pots and, at the same time, the pots were seeded to safflower. For comparison, a standard series was prepared by applying known amounts of the herbicides directly to the soil of pots with untreated thistle plants.

It was found that a phytotoxic amount of both dicamba and picloram was absorbed from the soil by the indicator plants as evidenced by leaf narrowing and twisting, and, in some instances, inhibition of leaf development. Since the safflower seedlings did not grow well, no accurate quantitative estimations could be made. A rough estimate is that approximately 0.5% of the foliar-applied chemicals was exuded from the roots of Canada thistle into the soil. The result for dicamba is comparable to Linder's estimate that less than 1% of a foliar-applied dosage of this herbicide was excreted from the roots of a bean plant during a 24 hour period following application (31).

VII. Bioassay of picloram translocation

For quantitative assessment of picloram translocation in Canada thistle, several bioassay methods with cucumber seedlings, bean (25), safflower, and sunflower plants were tried. Of the methods tried bioassay

with sunflower plants was the most satisfactory one.

In a series of preliminary tests sunflower seedlings were found very sensitive to picloram and they showed typical responses such as stem bending, wrinkling and declining of the leaves, swelling of the petiole and epicotyl, and stunting of plant growth. Minimum dosage required for plant response was less than 0.02 μg/plant for foliar application.

To test whether some components of Canada thistle themselves could affect sunflower plants, thus interfering with the results, ethanol extract of untreated thistle tissue was applied to the foliage of the indicator plants and no effect was observed.

Sunflower seeds were planted in 10-cm pots and grown in a growth chamber at 24°C. under a 16-hour light regime. The plants were grown for 10 days, at which time the cotyledons were partially expanded and the first pair of leaves was 0.5 - 1.0 cm in length. Uniform seedlings were selected and the pots were transferred to the greenhouse for use.

Ethanol extracts of picloram-treated Canada thistle plants were prepared as described earlier, and a 10 μl droplet of these extracts was applied to one cotyledon of a sunflower seedling. Three plants were used for each treatment. The sunflower seedlings treated with the assay solution were compared at various time intervals with a standard dosage series of 0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 μg/plant of picloram. Part of a standard dosage series is shown in Fig. 8.

The first symptom that appeared on the treated sunflower seedlings

Fig. 8. Part of a standard dosage series showing stem bending of sunflower seedlings as a response to picloram treatment. Dosages increase from left to right: 0, 0.1, 0.2, 0.4, 0.8, 1.6 µg/plant. Photographed eight hours after treatment.

was stem bending. Time of appearance and degree of stem bending in the standard series varied from 2.5 to 10 hours following application and from 0 to more than 90 degrees, depending on the concentration of picloram applied. With time, other symptoms occurred, and growth of the plants was inhibited progressively as the concentration of the chemical increased.

The sunflower plants were harvested four weeks after treatment, and the length of the first internode and the dry weight of the plant were determined. From the means of triplicate treatments in the standard series, dosage-response curves (Fig. 9) were made by plotting the measurements against concentration on semi-log paper (19). These dosage-response curves were used to estimate the concentration of picloram in the extracts. In general, the values obtained from the two dosage-response curves showed

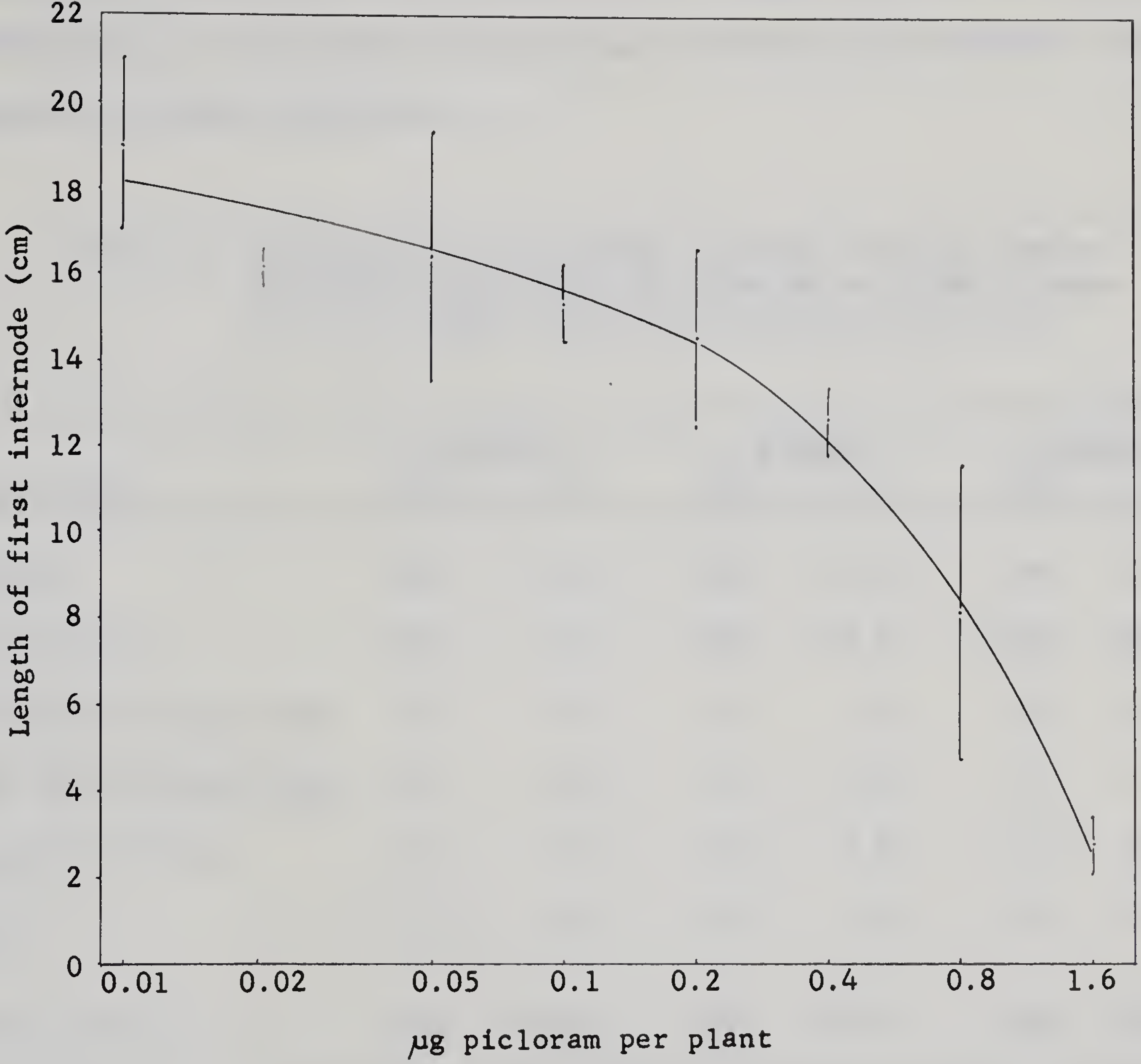


Fig. 9. Dosage-response curve of picloram on sunflower in standard series. Vertical lines in graph represent standard error of means.

good agreement and coincided with those estimated from the symptom comparison.

The estimates of the concentration of picloram from different comparisons and replicate plants were then averaged to give an estimated concentration of the chemical for each part of the thistle plants. The results are shown in Table II.

Table II. Distribution of picloram in Canada thistle plants following application of 1,000 µg in a 100 µl droplet to a single leaf about mid-way up the stem.

	1 day		3 days		9 days	
Plant parts	µg	%	µg	%	µg	%
Washing	620	75.3	120	17.2	24	4.7
Treated leaf	100	12.2	480	69.0	430	84.6
Part above treated leaf	33	4.0	13	1.9	24	4.7
Part below treated leaf	46	5.6	16	2.3	9	1.8
Untreated shoots	14	1.7	18	2.6	11	2.2
Roots	10	1.2	49	7.0	10	2.0
Total recovery	823	100.0	696	100.0	508	100.0

It is evident that after one day more than 75% of the recovered picloram was still on the surface of the treated leaf. About 25% had penetrated into the leaf and half of that moved out of the treated leaf into other parts of the plant. More than 80% had entered into the leaf three days after treatment; nearly all of it had penetrated into the leaf nine days following application. The amount of picloram in other parts of the

plant, however, no longer increased after one day. Total recovery of picloram decreased with time after application.

Two questions immediately arise: Why was there no increase after one day in the total amount of picloram in the other parts of the plant while the amount of picloram in the treated leaf greatly increased? Where did the rest of the chemical go?

A possible answer to the first question is that the transporting system of the treated leaf at or near the point of application was destroyed by the high dosage on a small leaf area (64 mm^2); thus there was no further export from the treated leaf. This indication agreed with the observation that application to a ring on a single leaf was less effective than application to four leaves (page 15 16). Another possibility is that the picloram did move into other parts of the plant but disappeared from there.

For the second question, there are several possible answers. Firstly, the picloram might have been broken down in the plants. This seems unlikely, because it was observed, in other experiments, that picloram could retain its phytotoxic activity in the plants for quite a long time. Secondly, the picloram could have been excreted by roots into the soil. Exudation of picloram from Canada thistle roots was indeed shown to occur, but the amount exuded was small, and besides, the picloram in the soil could be absorbed again by the root. Thirdly, since the volatility of picloram is high (15), it might be simply lost to the air directly from the treatment solution. This is unlikely, however, because the treatment solution was applied in a plastic ring which was covered immediately with a piece of parafilm. The fourth possibility is

that the picloram was bound to plant tissue components in such a way that it could not be extracted with ethanol.

VIII. Experiments with radioactive dicamba

Autoradiography

Plant mounts and autoradiograms in Fig. 10 show the distribution of radioactivity in plants at different times following application of 0.1 μc dicamba* (1.89 mc/mM) in a droplet on a single leaf. In the autoradiograms the dark image shows the location of the label whereas the faint images are due to excessive pressure in the plant mount-film pack during exposure of the x-ray film (10). The radioactive material that appeared in plant parts other than the treated leaf was primarily in the form of dicamba* as determined by chromatographic analysis of plant extracts (page 50). Therefore, the movement of the label in the plant represented mainly the translocation of the applied material, dicamba*.

It is clear from the autoradiograms that two hours following foliar application dicamba* started to move from the treated spot. Four hours after treatment, movement of the label within the treated leaf was evident, but no appreciable amount of it was exported from the leaf. In eight hours, some of the chemical moved symplasticly out of the treated leaf and was translocated both downward and upward in the treated shoot. Accumulation of the label in the growing tip of the treated shoot became more clear 16 hours after treatment. The label then also appeared in the root system. One day after application still more of it reached the young leaves and the roots, and it began to appear in the untreated shoot. At the end of the four-day period an appreciable amount of the tracer had

Fig. 10-1. Time course of translocation of dicamba* in Canada thistle plants which were treated with 0.1 μc of dicamba* (1.89 mc/mM) in a 10 μl droplet on a single leaf. Left: plant mounts. Right: autoradiograms. A. 2 hrs., B. 4 hrs., C. 8 hrs., D. 16 hrs.

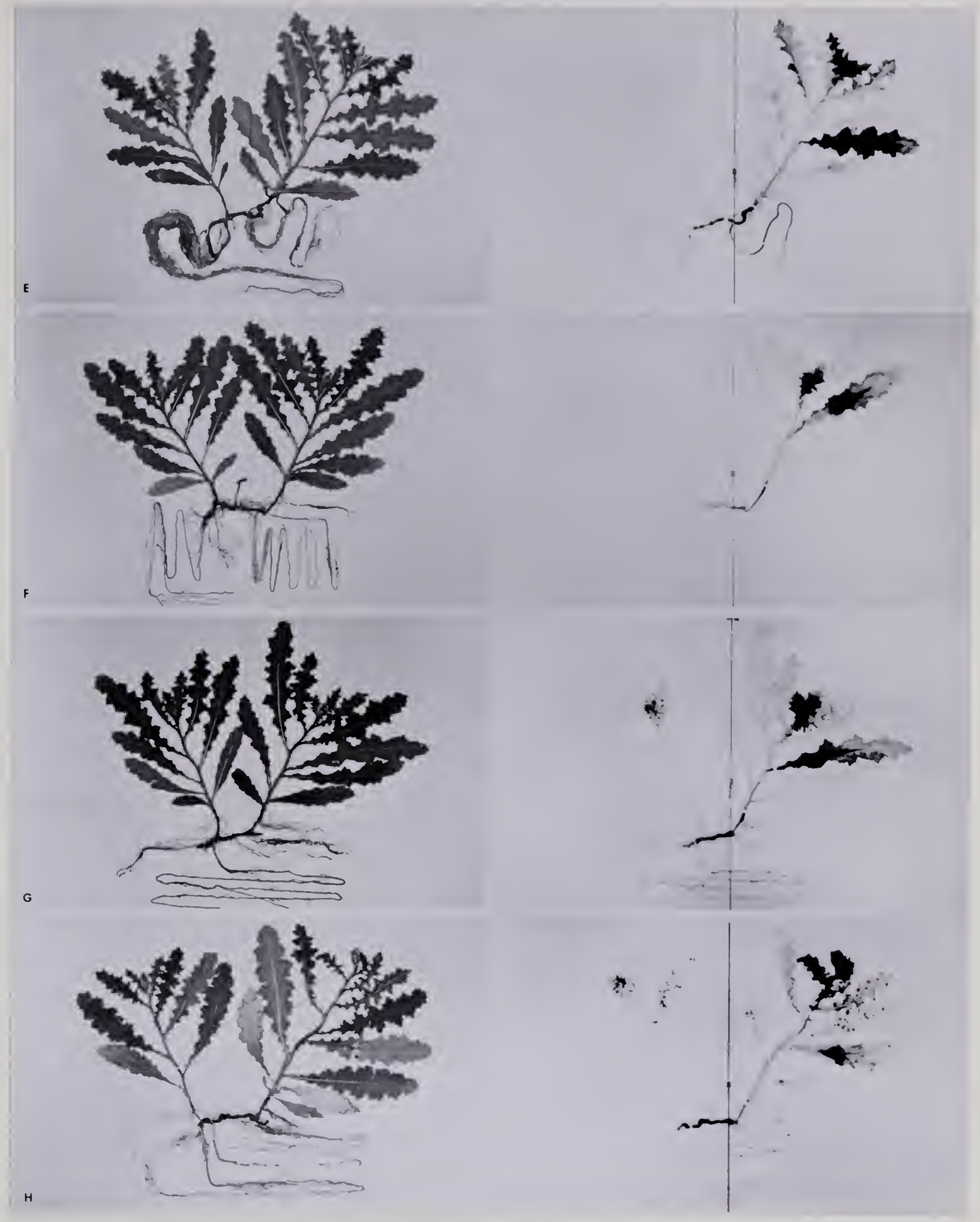


Fig. 10-2. Time course of translocation of dicamba* in Canada thistle plants which were treated with 0.1 µc of dicamba* (1.89 mc/mM) in a 10 µl droplet on a single leaf. Left: plant mounts. Right: autoradiograms. E. 1 day, F. 2 days, G. 4 days, H. 8 days.

accumulated in the growing tip of the untreated shoot, sufficient to give a clear image. Little change in the distribution pattern of the label occurred after four days. The radioactivity was still largely restricted to the treated leaf even at the end of the eight-day period.

The label also appeared in mature untreated leaves, but the density in these leaves was much less than that in the young growing leaves. When new sprouts were present, accumulation of the labeled material in these was greater than that in the older untreated shoot of the same plant (Fig. 11).

The accumulation of the label in young leaves and new sprouts indicates a source-to-sink pattern of translocation in the assimilate stream with food materials. The new sprouts are active sinks whereas old shoots are capable of manufacturing and exporting foods. Therefore, more of the labeled chemical was translocated to the new sprouts than to the untreated old shoots. This can explain the observation in a previous experiment in which new sprouts showed more response to the herbicide than did old shoots even if they were located much farther from the treated shoot than were the untreated old shoots.

Since some of the label also appeared in mature leaves, it appears that this compound migrated from phloem to xylem and was retransported to the mature leaves via xylem. According to Crafts et al. (10), the ability of a herbicide to leak from phloem to xylem relates to its ability to be exuded by roots. In cases where leakage into the transpiration stream occurs, leakage into the external medium of the roots also takes place. Root exudation of dicamba in Canada thistle plants was observed in a previous experiment (page 28).

Fig. 11. Mount (lower) and autoradiogram (upper) of a plant treated with 0.1 μc dicamba* (1.89 mc/mM) for 4 days showing accumulation of the label of dicamba* in new sprouts.

Autoradiograms of plants treated in nutrient solution (Fig. 12) indicate the rapid absorption through roots and upward movement of dicamba*. Within half an hour, the label in the roots gave a very clear image and a small amount appeared in the shoots. With time, more and more of the label entered the plants, and a substantial amount was present in the leaves after eight hours. From 16 hours on, accumulation of the radioactivity in the young leaves became evident. The rapid upward movement and distribution pattern indicate that, in contrast to the treated leaf, there was little retention of dicamba* in the roots. Judged from the rate of upward movement and the distribution in mature leaves, dicamba* was translocated mainly through the xylem following root absorption.

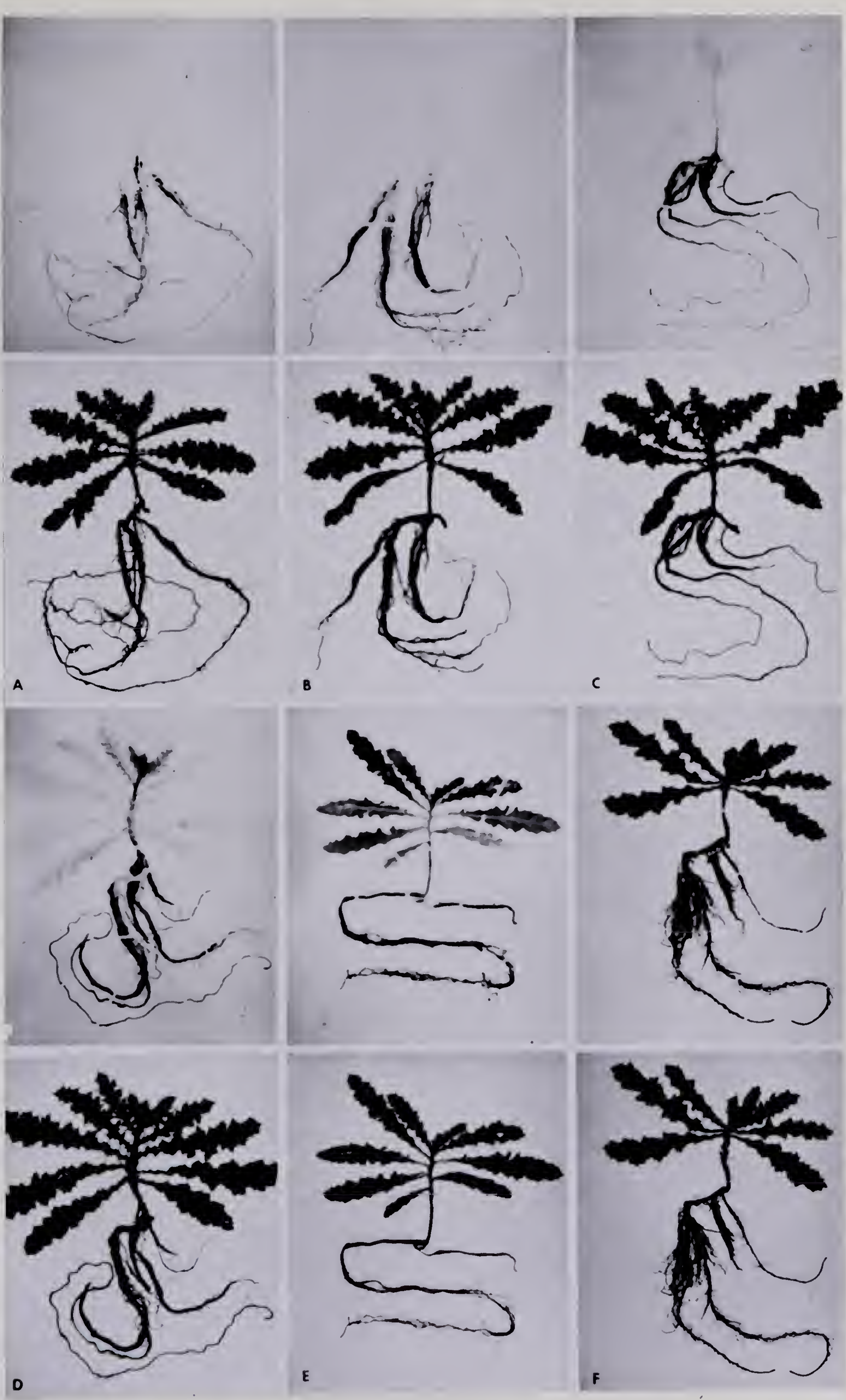
Accumulation of a root-applied compound in the developing young tissues is sometimes explained as a result of redistribution. The compound is translocated upwards via the transpiration stream and becomes evenly distributed in the shoot. Then it is retranslocated out of the mature leaves via the phloem into the rapidly developing tissues and accumulates there. Results of the work reported here provide no direct evidence for the occurrence of such a redistribution, but it cannot be ruled out as an explanation for the accumulation pattern observed.

Radioactivity extraction and counting

(a) Time course of translocation

Canada thistle plants were treated with 50 µl of a solution of dicamba* containing approximately 60 µg of the chemical. The dosage of radioactivity applied was 0.5 µc per plant, the equivalent of 360,000

Fig. 12. Time course of translocation of dicamba* in Canada thistle plants after root absorption from a nutrient solution of 100 ml containing 0.5 µc of dicamba* (1.89 mc/mM). Treatment period: A. 2 hrs., B. 4 hrs., C. 8 hrs., D. 16 hrs., E. 1 day, F. 4 days. The lettered photographs represent the plant mounts, the corresponding photographs above them the autoradiograms.


A
B
C
D
E
F

cpm in the gas flow counter used. The treatment times were 1, 3, 9 hours, 1, 3, 9, 27, and 54 days. Three plants were used in each treatment.

The treatment solution dried approximately 36 hours after application. Slight necrosis occurred on the treated area two days after treatment and became more severe with time, but the midrib remained green throughout the whole period.

The results of radioactivity determinations on extracts of different plant parts at the end of these treatment periods (Table III) indicate that penetration of the applied dicamba* into the leaf started within one hour, and continued for nine days. Some translocation of the labeled material out of the treated leaf had occurred three hours following treatment, but the activity of the samples at that time was only 2 - 5 cpm above background. Up to nine hours the amount translocated out of the treated leaf was still very small. After that the total radioactivity in the rest of the plant increased quickly, up to three days, remained nearly constant between three and nine days, and then gradually declined to roughly 25% of the three-day value. The percentage of the radioactivity that appeared in these plant parts was small, reaching a maximum of 16% at nine days, at which time 73% of the recovered radioactivity was in the treated leaf and 11% in the residue on the leaf surface (Fig. 13).

The distribution of the label in different parts of the plants is graphically illustrated in Fig. 14 as percentages of the total amount recovered at each time interval. It is recognized that loss of radioactivity from the plant parts may have occurred, but the viewpoint is

Table III. Distribution of radioactivity in Canada thistle plant at various time intervals following application of 0.5 μc dicamba* (1.89 mc/mM) to a single leaf (Standard = 360,000 cpm). Results are averages of 3 replicates and are expressed as cpm.

Time	Plant part								Recovery (%)
	Washing	Treated leaf	Upper* part	Lower* part	Untreated shoot	Roots	New sprouts**	Total	
1 hr.	312,600	7,390	0	0	0	0	-	319,990	88.9
3 hr.	281,130	23,890	80	80	47	53	-	305,280	84.8
9 hr.	240,200	62,960	230	360	107	573	-	304,370	84.6
1 day	187,470	121,970	4,290	1,613	627	4,040	-	320,010	88.9
3 days	74,200	156,930	25,733	4,853	780	9,607	-	272,103	75.6
9 days	21,710	158,570	23,460	1,480	6,120	3,440	610	215,390	59.8
27 days	20,800	145,600	10,453	947	2,760	933	2,093	183,586	51.0
54 days	20,250	139,970	6,887	233	1,480	327	1,620	170,767	47.4
L.S.D. (5%)	8,810	17,590	3,120	600	1,420	1,590	540		

* Relative to treated leaf.

** There were no new sprouts yet 3 days after treatment.

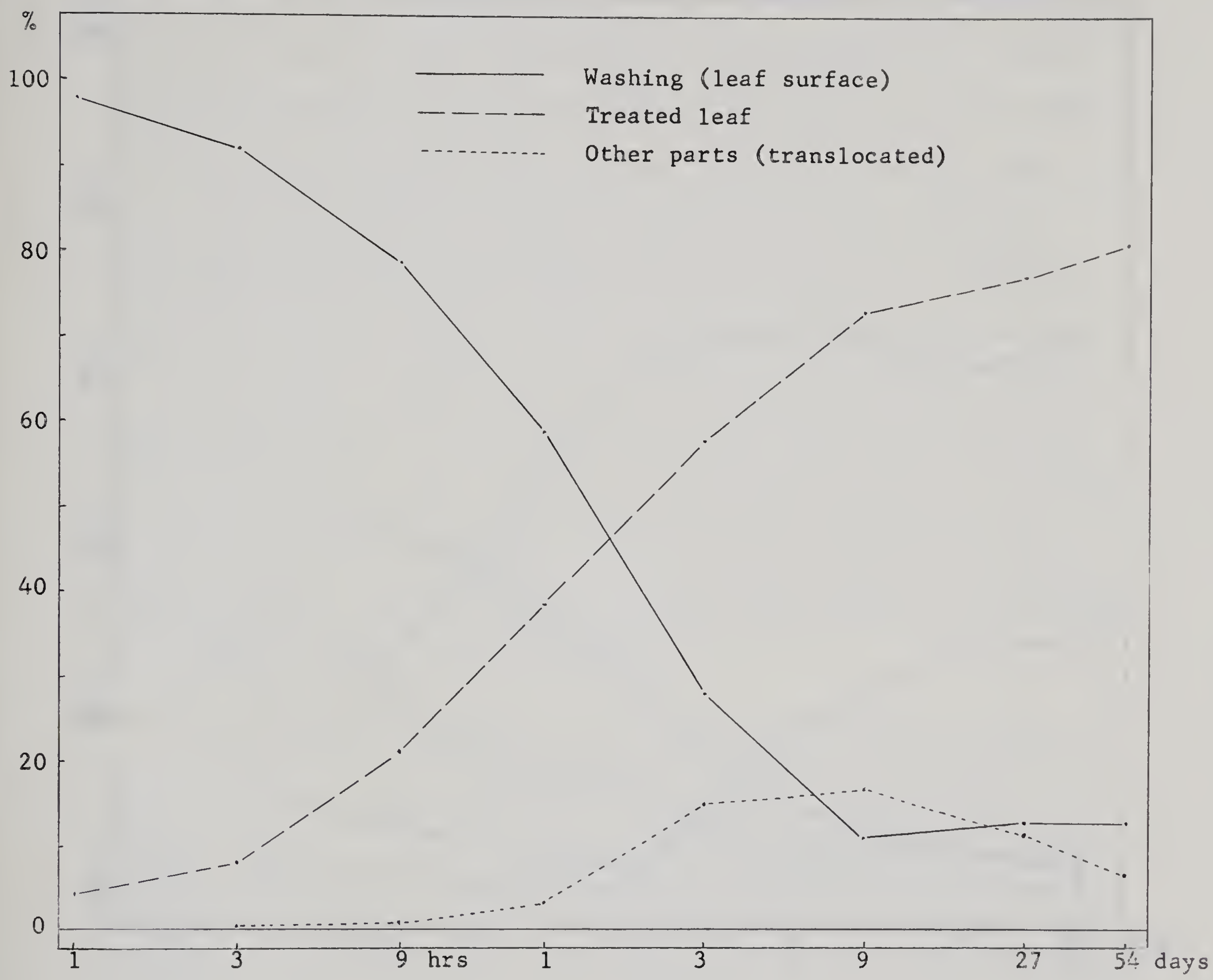


Fig. 13. Distribution of radioactivity in washing (leaf surface), treated leaf, and other parts of the plant (pooled data) at various time intervals following application of 0.5 µc of dicamba* (1.89 mc/mM) to a single leaf of Canada thistle plants. The percentages are calculated from Table III, taking the recovered counts at each time interval as 100 per cent.

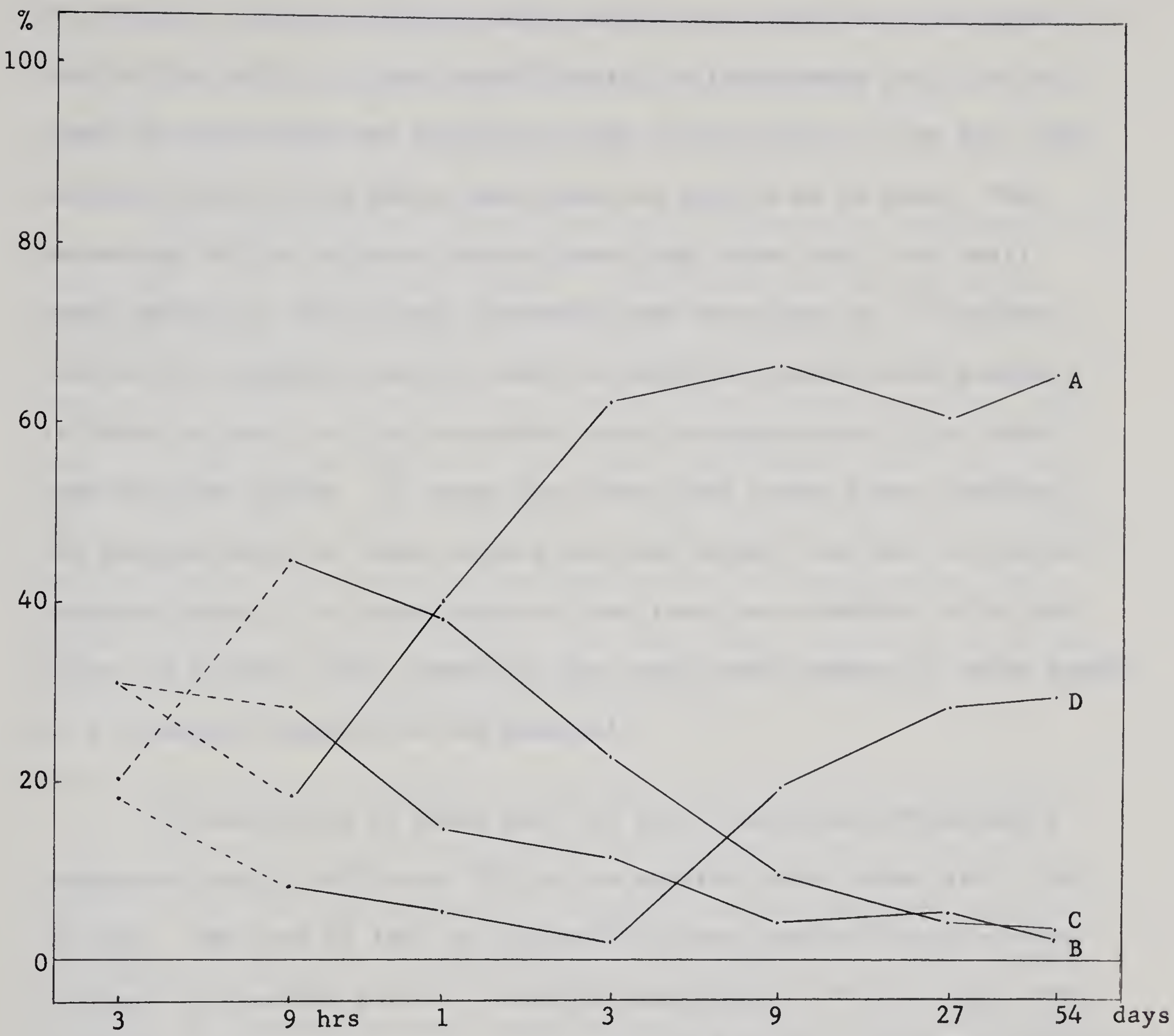


Fig. 14. Distribution of translocated C^{14} in Canada thistle plants at various time intervals following application of dicamba* to a single leaf, expressed as percentages of the total counts present in these plant parts at each time interval. A - upper part of treated shoot. B - lower part of treated shoot. C - roots. D - untreated shoots.

taken that the greater the activity present in the various plant parts, the greater was the amount of radioactive material translocated to them. The results indicate that the label tended to accumulate in the upper part of the shoot, or, more specifically, in the growing tip. In the roots the percentage was relatively high at nine hours to one day, then declined quickly from 45% at nine hours to only 3% at 54 days. The percentage in the untreated shoots (one large shoot and a few small young sprouts on each plant) increased from three days on. Translocation of the chemical from the roots to untreated shoots could account, at least in part, for the relatively rapid disappearance of the label from the root system. In every case when young sprouts were involved, the radioactivity in these sprouts was much higher than that in the old untreated shoot. No accumulation of the label was observed in the part below the treated leaf, especially the stem, which appears to serve simply as a transport channel for the chemical.

It should also be noted that the total recovered radioactivity decreased rapidly with time, 53% of the applied counts being lost after 54 days. The rate of loss of radioactivity was especially high during the one- to nine-day period. Possible explanations for this high rate of loss are almost the same as those suggested in the picloram experiment, i.e. the herbicide may be broken down in the plants, excreted from the roots, lost by evaporation, or bound tightly to plant tissue components. However, some points have been further clarified with the radioassay technique.

During the 9- to 54-day period, the activity in the washing remained nearly constant, though 12% of the applied counts were lost during

this time. Therefore, the loss of chemical by direct evaporation of the treatment solution from the leaf surface could, at least after nine days, not be considered an important factor. In fact, the loss of label during the last 45 days was mainly from the parts of the plants other than the treated leaf.

The possibility that the radioactive material was conjugated with some plant constituents was studied. After the first extraction the residues of the samples of the 9-day treatment were hydrolyzed with 0.1 N hydrochloric acid for 24 hours and further extracted with ethanol, methanol, and water four times. A total amount equivalent to 1.0 - 1.7 per cent of the applied dose of radioactivity was recovered from the extracts in this manner, indicating that dicamba* did not conjugate with plant constituents to any great extent. The amount recovered does not account for the 40% loss of radioactivity in nine days.

Some metabolic change or breakdown of the herbicide (discussed later, page 49) did occur in the tissue, but such change should not result in the loss of radioactivity unless a radioactive gaseous product was produced during the breakdown process. Radioactive carbon dioxide released by buckwheat plants treated with dicamba* has been detected by Vanden Born (42).

The next question is why, after three days, there was no increase, but rather a rapid decrease, in the amount of radioactivity in the rest of the plant while the activity in the treated leaf remained high? It is possible that the rate of disappearance of activity from these plant tissues was higher than the rate of import of the chemical from the treated leaf. Actually the rate of export of the chemical from the

treated leaf to other tissues was very low after nine days. During the last 45 days the net loss of activity from washing was 1,460 cpm, and that from the treated leaf was 18,600 cpm. Even if all this activity was transported to the rest of the plant, it was still much less than the amount translocated in the first three days. The slowdown or ceasing of export of the chemical is probably due to disturbance of or injury to the translocating system of the treated leaf by the herbicide. It is also possible that the entire leaf tissue was damaged so that insufficient photosynthate was produced; without production and export of assimilate, translocation of the chemical out of the leaf would probably not take place then.

(b) Effects of dosage on translocation

Dicamba* at dosages of 0.1, 0.5, and 1.0 μc per plant was applied to a single leaf (three plants per treatment), and three days after application the plants were harvested and the radioactivity in the extracts from different plant parts was determined. The results given in Table IV show the radioactivity in different parts of the plants as percentages of the applied dosage. In general, in this dosage range, the activity in the extracts increased with the dosage, but the proportion exported from the treated leaf decreased when the dosage was increased.

To obtain a wider dosage range, unlabeled dicamba was added to the treatment solution containing 0.1 μc of dicamba*. The results (Table V) again show that high dosage decreased the proportion exported. Pretreatment with unlabeled dicamba inhibited both penetration and translocation of the labeled material whereas post-treatment showed

Table IV. Effect of dosage on the translocation of dicamba* in Canada thistle plants. Results show activity in extracts of the various plant parts as percentages of the applied counts three days after application to a single leaf.

Plant part	Dosage (μc) 0.1	 0.5	 1.0*
(1) Washing	5.8	20.6	36.6
(2) Treated leaf	39.7	43.6	35.8
(3) Upper part	12.0	7.2	1.6
(4) Lower part	1.4	1.3	0.4
(5) Roots	2.9	2.6	0.8
(6) Untreated shoot	1.0	0.2	1.0
Total	62.8	75.6	76.3
Total exported from treated leaf: (3) + (4) + (5) + (6)	17.3	11.3	3.8

* Results in this column are means of two treatments only.

Table V. Effect of application of unlabeled dicamba on the export of C^{14} from dicamba* at 0.1 μc (approximately 12 μg). Unlabeled and labeled material were applied to the same spot on a single leaf. Plants were harvested three days after treatment.

Fraction	μg dicamba added 0	 10	 100	 1000	100 μg 1 day pretreatment	100 μg 1 day post treatment
cpm penetrated	41,080	39,572	42,240	43,060	34,026	42,540
cpm exported	12,519	7,266	3,400	1,400	2,759	9,773
% exported	17.3	10.1	4.7	1.9	3.8	13.6

relatively little effect.

It has been postulated (7) that a high dosage, resulting in contact injury, might inactivate the phloem transport system and hence prevent systemic distribution. In this work, the phloem transport system apparently was not inactivated completely, but some degree of damage was possible. It is also possible that the phloem transport system was saturated. After being saturated or nearly saturated transport of herbicide would no longer increase with dosage and the proportion transported would decrease with increasing dosage.

Metabolism of dicamba* in Canada thistle

It was noticed that following application to leaves of Canada thistle plants, radioactivity in the plant extracts decreased rapidly with time (page 45), and the loss of the activity was thought perhaps partially due to the breakdown of the chemical in the plant tissue. The main concern of this series of experiments was, therefore, to investigate the possible metabolism of this chemical following foliage application.

Small amounts of the extracts prepared for radioactivity determination were applied as bands on 4-cm strips of Whatman No. 1 chromatography paper and dried by a stream of warm air. If the radioactivity in the extracts was too low, the extracts were further evaporated down to smaller volumes. The volumes applied to each strip varied from 30 to 500 μl to give an approximate activity of 500 - 8,000 cpm. The chromatograms were developed, using the descending technique, in isopropanol:ammonium hydroxide (28%):water, 8:1:1, and the solvent was allowed to run 30 cm.

Radioactive regions of chromatograms were detected using a Nuclear-Chicago Actigraph II chromatogram scanner. Autoradiograms were also made on Ansco non-screen x-ray film for varied exposure time depending on activity level. To obtain more accurate quantitative information, radioactive zones on the chromatograms, located by reference to the autoradiograms, were cut out and eluted with ethanol and the activity in eluates was assayed using a gas flow counter.

Scans of the chromatograms showed that distribution of radioactivity from dicamba* solution was a single peak at a mean Rf 0.67. The active material extracted from the treated leaf ran as two peaks; in addition to the peak at the dicamba position, substantial amounts of C^{14} were present in a compound running at a mean Rf 0.87 (Fig. 15). For the extract from the tissue above the treated leaf, only a very low second peak was observed, and there was no indication of the presence of metabolic product in the extracts from other parts of the plant.

The results from autoradiograms of the chromatograms correspond to those obtained from the radiochromatographic scanning method. In all extracts distinct bands corresponding to dicamba* were detected. In the extracts of the treated leaf of three days or longer, a second band of higher Rf value was clearly observed. In other extracts no second band was detected on the autoradiograms, but in the extract from the plant part above treated leaf traces of activity in this Rf region were obtained from the gas flow counter. The distribution of radioactivity in the chromatograms is shown in Table VI.

It is evident that metabolic breakdown of dicamba* occurred in the plant. A new labeled product was produced from the applied dicamba*

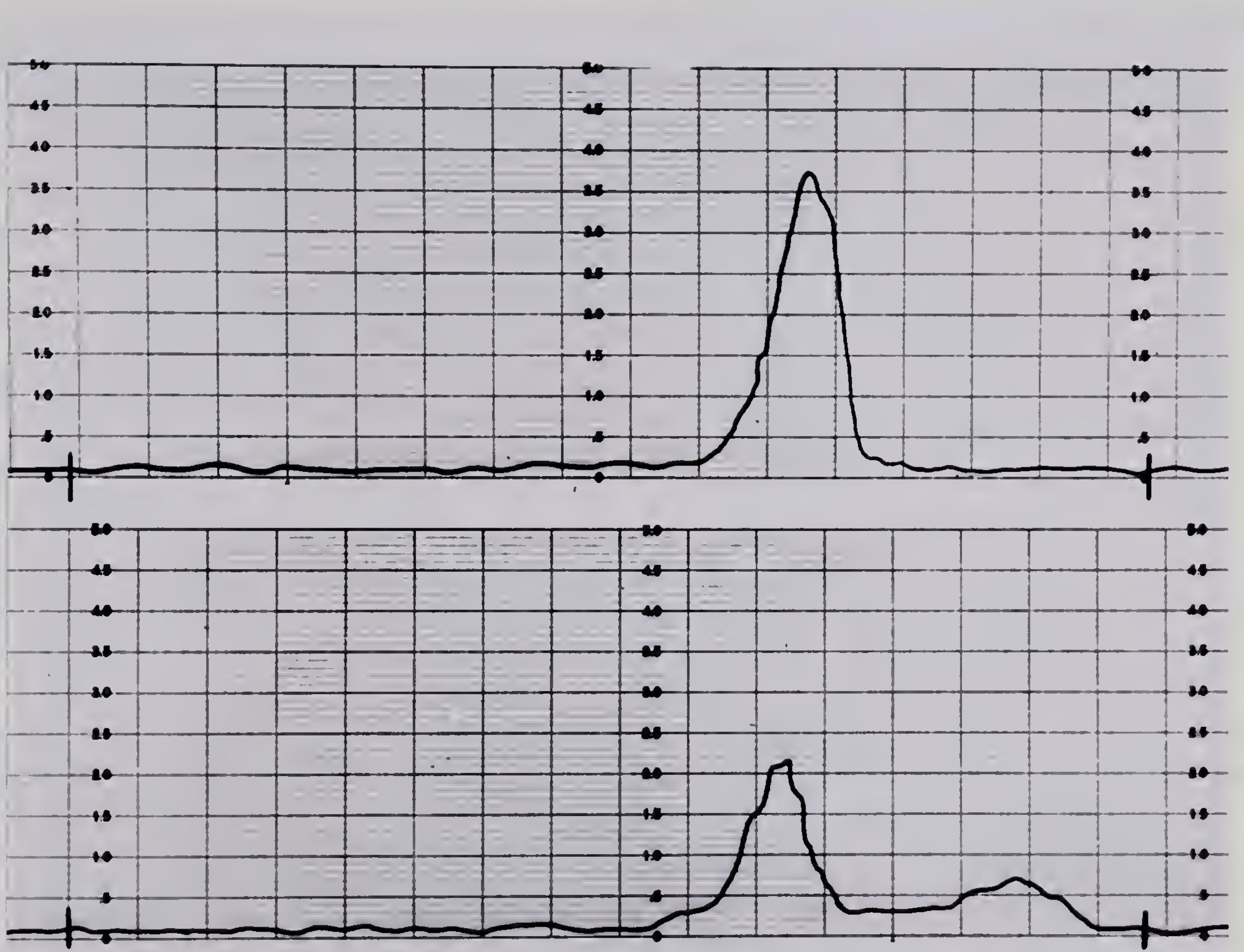


Fig. 15. Distribution of radioactivity along chromatograms of pure dicamba* (upper) and ethanol extract from the treated leaf of Canada thistle nine days after application of dicamba* as a droplet on the upper surface (lower). Chromatographic solvent: iso-propanol-ammonia-water (8:1:1). Origin is marked at left, solvent front at right.

Table VI. Distribution of radioactivity along chromatograms of ethanol extracts from Canada thistle tissues following application of dicamba* to a single leaf, as determined by measuring the activity in the eluates of the radioactive zones on the chromatograms in a gas flow counter. Chromatographic solvent: _iso_propanol-ammonia-water (8:1:1).

Tissue extracted	Percentage of radioactivity Rf 0.60 - 0.74 (mean Rf 0.67)	 Rf 0.80 - 0.94 (mean Rf 0.87)
Dicamba* (treatment solution)	100	0
Treated leaf, 1 day	96.6	3.4
Treated leaf, 3 days	83.5	16.5
Treated leaf, 9 days	78.5	21.5
Treated leaf, 54 days	63.1	36.9
Upper part, 9 days	97.0	3.0

and its proportion increased with time up to 21.5% of the total recovered amount after nine days and 36.9% after 54 days in the treated leaf. It is of interest that, after nine days, this metabolite in the extract of the tissue above the treated leaf accounted for only 3% of the total activity, and that in the extracts from other parts of the plant it could not be detected. It appears that the breakdown of dicamba* took place mainly in the treated leaf and that this metabolic product was translocated less readily than was the original chemical. It is also possible that this metabolite was further degraded in the plant tissue and that $C^{14}O_2$ was lost to the surrounding atmosphere and hence could not be detected in the chromatograms. If the former is the case, the question arises why the metabolism of dicamba* occurred much more readily in the

treated leaf than in the other tissues. If the latter is true, the problem of the rapid loss of radioactivity will be solved. Without direct evidence, however, we cannot draw further conclusions.

CONCLUSION

To kill deep-rooted perennial weeds a herbicide must reach the roots in sufficient amounts to bring about death of the cells capable of producing shoot buds (7). The results of the experiments reported here indicate that both dicamba and picloram possess this character; they were readily absorbed both by foliage and by roots, and translocated in the phloem and xylem in Canada thistle plants. After application to the leaves they were translocated into the stem, then into the roots, and finally exuded from roots of the treated plants into soil in amounts sufficient to affect safflower seedlings growing in the same soil.

Application of the herbicides to mature leaves resulted in more extensive and more rapid translocation than did application to the young leaves in the shoot tips. Injury symptoms were most severe in the young developing leaves and the new sprouts after application to the mature leaves. All these observations support the suggestion of other workers (e.g. 6, 10) that phloem movement of herbicide proceeds generally from source of assimilates to the sink where assimilates are being utilized in growth. Mature leaves are the source of assimilates, being capable of manufacturing and exporting foods, whereas young expanding leaves and sprouts are active sinks which need to import food materials for development.

Arny in 1932, as reported by Crafts and Robbins (8), found that the readily available carbohydrate reserves in the underground parts of Canada thistle plant varied with the season and reached a minimum during the latter part of June and the first part of July, the beginning bloom stage of this species. Bakker (3) also reported that carbohydrate reserves

in Canada thistle plants were low in the early flowering stage, in early summer. Theoretically in the early flowering stage, when the organic reserves in the roots are low and photosynthesis in the shoots is high, there should be active source-sink movement of assimilates, and this should result in maximum translocation of herbicides. However, the present experiments in the field did not show any one stage of plant development to be optimal for translocation of these two herbicides. This might be due to interference by other factors, e.g., the weather. Owing to the low temperature and insufficient moisture in the field after planting, the plants did not develop rapidly during the early summer. When they reached the early flowering stage in September it was cold already; absorption and translocation do not occur as readily under low temperature as they do under higher temperature (36).

The results of quantitative assessments were, in general, in agreement with the observations of physiological responses. One discrepancy between them is that the amount of picloram recovered from the plant parts other than the treated leaf no longer increased after one day as determined by bioassay method, while the gradual mutilation studies showed that export of picloram from the treated leaf continued for more than three days. It is possible that export of picloram out of the treated leaf continued to occur after one day, but that the chemical disappeared after being transported into the other parts of the plant or became bound to some plant constituents and thus could not be detected with the bioassay method.

By comparing the injury symptoms of the plant tissues and the amounts of herbicides detected in those tissues it is found that very

small amounts of the chemicals could bring about injury to the plant, e.g., 0.03 μg of dicamba in the shoot might result in discoloration of the young developing leaves and 10 μg of it might cause death of the growing point of the shoot.

Autoradiograms of the plants treated with dicamba* indicated clearly the location of the label; the intensity of the image correlated closely with the counts of radioactivity in the plants. Following foliar application a dense image was shown in the treated leaf, especially on the application spot. Accumulation of the label in the young expanding leaves and the new sprouts was apparent, and this type of accumulation was in agreement with the observation of physiological responses in that this herbicide, as well as picloram, caused most severe injury to the young growing tissues of the plants. When absorbed by the root, dicamba* was distributed rapidly throughout the plant with some accumulation in the young leaves. In contrast to the leaf, there was little retention of dicamba* in the roots. This is probably one of the reasons why soil application of dicamba showed more killing effect on plants than did foliar application.

In general, dicamba and picloram showed similar behavior in translocation in Canada thistle plants, but there was some variation in the responses between the two herbicides on the plants. Picloram caused faster stem bending and killing of the treated shoot whereas dicamba showed quicker effects on the untreated shoots. Picloram treatment caused serious swelling of the creeping roots, but no such swelling was observed in the dicamba treatment. These observations might suggest that there are some differences in the pattern of distribution between the two

herbicides, but the results of quantitative determinations do not support this suggestion. The variation in the physiological responses on this species might be simply the difference in sensitivity of the plant tissues to the two chemicals.

Metabolic change of dicamba was detected in Canada thistle plants. Since the metabolite was detected mainly in the extract from the treated leaf, it seems that the change occurred in the treated leaf and the metabolite was less readily translocated than the intact dicamba, or that it was further degraded and lost to the air more readily in other parts of the plant than in the treated leaf. This is a question which requires further study.

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